

What is claimed is:

- add E1*
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1. A set of synthetic oligonucleotides consisting of a 5' and 3' oligonucleotide for polymerase chain reaction of hepatitis B virus, wherein the 5' oligonucleotide is a 14mer which contains a mutation leading to amino acid changes in human hepatitis B virus proteins leading to escape viral mutants, including those derived from immunoprophylaxis with vaccines and hepatitis B immune globulin and from treatment with antiviral drugs and the 3' oligonucleotide with an appropriate size which is conserved between the mutant and wild type strains.
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2. The oligonucleotides of claim 1, wherein the 5' oligonucleotide *(SEQ ID NO: 1)* has the sequence, 5'-TACGGACAGAACT-3', which corresponds to position 582 to 595 in the wild type human hepatitis B virus genome and contains the mutation G to A, leading to change at amino acid 145 of hepatitis B virus surface antigen from Glycine to Arginine, at position 8 of the said oligonucleotide.
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- 25 3. The oligonucleotide of claim 1, wherein the 3' oligonucleotide *(SEQ ID NO: 2)* has sequence, 5'-TTAGGGTTTAAATCTATACCC-3', which corresponds to position 842 to 822 in the wild type human hepatitis B virus genome and is complementary to the coding strand of human hepatitis B virus surface antigen.
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- 35 4. A composition comprising an amount of the oligonucleotides of the claim 1, 2 or 3 suitable for polymerase chain reaction.
5. A method of determining the presence of human

hepatitis B virus surface antigen mutant 145
(Glycine to Arginine) in a sample comprising:

- 5 a) obtaining the sample;
 b) treating the obtained sample for uses in a
 polymerase chain reaction; and
 c) amplifying the treated sample with the two
 oligonucleotides in claim 1, 2 or 3, wherein
 if the sample is amplified will indicate that
10 the sample contains human hepatitis B virus
 surface antigen mutant 145 (Glycine to
 Arginine).

15 6. The method of claim 5, wherein the sample is a
 serum sample.

 7. An oligonucleotide of appropriate size, containing
 a mutation leading to amino acid changes in human
 hepatitis B virus proteins leading to escape viral
 mutants, including those derived from
20 immunoprophylaxis with vaccines and hepatitis B
 immune globulin and from treatment with antiviral
 drugs, wherein the 5' terminus of the
 oligonucleotide is with a fluorescent dye,
 6-(fluorescein-6-carboxamido) hexanoate (6FAM); the
25 3' terminus is a primary amine group; and a poly-T
 linker consisting of 12 T is preceding the 3'
 primary amine group.

30 8. The oligonucleotide of claim 7, wherein the length
 of the oligonucleotide corresponding to the viral
 genome is 14 to 20 nucleotides.

35 9. A set of oligonucleotides useful for immobilizing
 on solid supports, wherein each pair of
 oligonucleotides consist of:

- A. a first oligonucleotide having 27 nucleotides
 with a first fluorescent dye, at its

5' terminus and a primary amine group at its 3' terminus: 5'-(6FAM)TACGGACGGAAACTGTTTTTTTTTTTTT (C-7 amine)-3'; wherein the first fifteen nucleotides correspond to wild type human hepatitis B virus genome position 580 to 594, and the poly-T is a synthetic linker aiming at facilitating the subsequent hybridization reaction with target viral DNA sequences from serum samples; and

B. second oligonucleotide having 27 nucleotides with a second fluorescent dye at its 5' terminus with a sequence, 5'-dye-TACGGACAGAAACTGTTTTTTTTTTTC-7 amine-3'; wherein the first fifteen nucleotides contain the mutation G to A at position 8 of the said second oligonucleotide, in bold, leading to change at amino acid 145 (Glycine to Arginine) of human hepatitis B virus surface antigen, and correspond to (wild type human hepatitis B virus genome position 580 to 594) and wherein the poly-T is a synthetic linker aiming at facilitating the subsequent hybridization reaction with target human viral DNA sequences from serum samples.

10. The synthetic oligonucleotides of claim 9, wherein the first and the second fluorescent dye is the same.

11. The synthetic oligonucleotides of claim 10, wherein the dye is 6-(fluorescein-6-carboxamido) hexanoate (6FAM).

12. A method to screen for human hepatitis B virus surface antigen mutant 145 (Glycine to Arginine), wherein the specific oligonucleotides in claim 9

with chemical modifications: a fluorescent dye at 5' terminus and primary amine group at 3' terminus, are immobilized on solid supports.

- 5 13. A method to screen for human hepatitis B virus mutants, derived either from immunoprophylaxis with hepatitis B immune globulin and vaccines or from treatment with antiviral drugs, or from asymptomatic hepatitis B virus carriers, wherein the specific oligonucleotides in claim 9 with chemical modifications: a fluorescent dye at 5' terminus and primary amine group at 3' terminus, are immobilized on solid supports.
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- 15 14. The method of claim 12 or 13, wherein the solid supports are glasses.
- 20 15. A set of oligonucleotides useful as amplifier probes in a polymerase chain reaction for human hepatitis B viral DNA, wherein the size of generated amplification product should be under 150 base pairs; wherein the generated amplification product should cover the said mutation site and in particular the G to A mutation leading to amino acid Glycine to Arginine change at position 145 of human hepatitis B virus surface antigen; wherein said oligonucleotides comprise at least two different oligonucleotides probes which are:
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- 30 A. first oligonucleotide having 20 nucleotides with a biotin group at its 5' terminus, 5'-Biotin-AGGATCAACAACAACCGTA, position 489 to 508 as referred to the wild type human hepatitis B virus genome, wherein the presence of a biotin group allows the separation of amplified DNA fragments using streptavidin magnetic particles; and
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(SEQ ID NO. 6)

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B. second oligonucleotide having 20 nucleotides with a fluorescent dye Texas Red at its 5' terminus, and complementary to the coding strand of human hepatitis B virus surface antigen with the sequence, 5'-Texas red-ATCGTCCTGGGCTTTCGCAA-3', position 634 to 615 as referred to the wild type human hepatitis B virus genome.

16. A method to amplify target DNA of human hepatitis B virus surface antigen from serum samples, by polymerase chain reaction using the oligonucleotides in claim 15 that contain modifications: addition of biotin and Texas red groups for sense and anti-sense oligonucleotides respectively, at their 5' terminus.

17. A set of oligonucleotides useful as amplifier probes in a polymerase chain reaction for human hepatitis B viral DNA, wherein the size of generated amplification product should be under 150 base pairs; wherein the generated amplification product should cover mutations that result in amino acid changes in human hepatitis B virus proteins leading to escape viral mutants, including those derived from immunoprophylaxis with vaccines and hepatitis B immune globulin and from treatment with antiviral drugs; wherein said oligonucleotides comprise at least two different oligonucleotides probes which are:

- A. a first oligonucleotide having 20 nucleotides with a biotin group at its 5' terminus, wherein the presence of a biotin group allows the separation of amplified DNA fragments using streptavidin magnetic particles; and
- B. a second oligonucleotide having 20 nucleotides

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